July 12, 2004

Laurie A. Miller
Workgroup Manager
The American Chemistry Council
Pyridine and Pyridine Derivatives Workgroup
1300 Wilson Boulevard
Arlington, VA 22209

Dear Ms. Miller:

The Office of Pollution Prevention and Toxics is transmitting EPA's comments on the robust summaries and test plan for Pyridine and Pyridine Derivatives Category posted on the ChemRTK HPV Challenge Program Web site on January 28, 2004. I commend The American Chemistry Council Pyridine and Pyridine Derivatives Workgroup for its commitment to the HPV Challenge Program.

EPA reviews test plans and robust summaries to determine whether the reported data and test plans will provide the data necessary to adequately characterize each SIDS endpoint. On its Challenge Web site, EPA has provided guidance for determining the adequacy of data and preparing test plans used to prioritize chemicals for further work.

EPA will post this letter and the enclosed comments on the HPV Challenge Web site within the next few days. As noted in the comments, we ask that the Pyridine Workgroup advise the Agency, within 90 days of this posting on the Web site, of any modifications to its submission. Please send any electronic revisions or comments to the following e-mail addresses: oppt.ncic@epa.gov and chem.rtk@epa.gov.

If you have any questions about this response, please contact Richard Hefter, Chief of the HPV Chemicals Branch, at 202-564-7649. Submit questions about the HPV Challenge Program through the "Contact Us" link on the HPV Challenge Program Web site pages or through the TSCA Assistance Information Service (TSCA Hotline) at (202) 554-1404. The TSCA Hotline can also be reached by e-mail at tsca-hotline@epa.gov.

I thank you for your submission and look forward to your continued participation in the HPV Challenge Program.

Sincerely,

-S-

Oscar Hernandez, Director
Risk Assessment Division

Enclosure

cc: W. Penberthy
M. E. Weber

EPA Comments on Chemical RTK HPV Challenge Submission:
Pyridine and Pyridine Derivatives

Summary of EPA Comments

The sponsor, the American Chemistry Council's Pyridine and Pyridine Derivatives HPV Work Group, submitted a test plan and robust summaries to EPA for the pyridine and pyridine derivatives category with a cover letter dated December 17, 2003. EPA posted the submission on the ChemRTK HPV Challenge Web site on January 28, 2004.

EPA has reviewed this submission and has reached the following conclusions:

1. Category Justification. Significant differences among the chemical structures and properties as well as the lack of evidence of similar mammalian toxicities argue against the proposed category. For these comments, EPA has grouped the chemicals as Piperidine, Alkylpyridines, Pyridinium chlorides, and Pyridinenitriles.

2. Physicochemical Properties. The submitter needs to provide measured vapor pressure data for 3-picoline, nicotinonitrile, and picolinonitrile and measured water solubility data for nicotinonitrile and picolinonitrile.

3. Environmental Fate. The submitter needs to provide measured ready biodegradation data for piperidine, picolinonitrile, nicotinonitrile, pyridine, alkyl derivatives, and 1-phenylmethylpyridinium, Et Me derivatives, chlorides ("pyridinium chlorides"). The submitter needs to provide photodegradation and fugacity data for pyridine, alkyl derivatives, and pyridinium chlorides. Even though EPA agrees with the submitter that these chemicals are stable in water and do not hydrolyze, the submitter needs to provide this information in the robust summaries and not only in the test plan.

4. Health Effects. The submitter proposes that tests according to OECD TG 421 be conducted on pyridine and on nicotinonitrile. Because some data are available for nicotinonitrile and because acute toxicity testing indicates greater toxicity of picolinonitrile compared with nicotinonitrile, EPA recommends that the submitter conduct a repeated-dose/developmental/reproductive toxicity screening test on picolinonitrile instead of nicotinonitrile for the Pyridinenitriles. In addition, EPA recommends that the submitter conduct the pyridine test on mice because of the greater sensitivity to pyridine of mice versus rats. To address deficiencies for piperidine, the submitter needs to conduct a repeated-dose/developmental/ reproductive toxicity screening study and testing on chromosomal aberrations. To address deficiencies for pyridinium chlorides, the submitter needs to conduct a repeated-dose/developmental/ reproductive toxicity screening study and genetic toxicity studies on pyridinium chlorides.

EPA reserves judgement on using the read-across approach within the Alkylpyridines group for pyridine, alkyl derivatives pending additional composition information on the this substance. EPA reserves judgement on the gene mutation data for piperidine and the acute toxicity data for pyridine, alkyl derivatives, and pyridinium chlorides, pending additional information. If these issues are not adequately resolved, additional testing may be needed for these chemicals.

5. Ecological Effects. The submitter needs to provide data on the following chemicals for all ecological endpoints: 1) piperidine; 2) pyridine, alkyl derivatives; 3) pyridinium chlorides; and 4) picolinonitrile.

The submitter provided adequate data for acute fish toxicity for picolinonitrile; however, daphnia and algae tests need to be conducted. For piperidine and pyridinium chlorides, all endpoints need to be tested. For pyridine, alkyl derivatives, an algal test needs to be conducted. Also, for this chemical, detailed information on the test substance composition and purity in the fish and daphnia studies is essential for determining the adequacy of data. Otherwise, tests on these endpoints need to be conducted .

EPA requests that the submitter advise the Agency within 90 days of any modifications to its submission.

EPA Comments on the Pyridine and Pyridine Derivatives Category Challenge Submission

Category Definition

The submitter defines the category as the following nine substances: piperidine (CAS No. 110-89-4), pyridine (CAS No. 110-86-1), 4-picoline (CAS No. 108-89-4), 3-picoline (CAS No. 108-99-6), 2-picoline (CAS No. 109-06-8), pyridine, alkyl derivatives (CAS No. 68391-11-7), 1-(phenylmethyl)pyridinium, Et Me derivatives, chlorides (CAS No. 68909-18-2), nicotinonitrile (CAS No. 100-54-9), and picolinonitrile (CAS No. 100-70-9).

The submitter needs to provide additional information on the composition and purity of 1-(phenylmethyl)-pyridinium, Et Me derivatives, chlorides ("pyridinium chlorides") and pyridine, alkyl derivatives.

Category Justification

The submitter justifies the category by claiming similarities of all members in structural and functional features, physical properties, biodegradability, environmental fate, mammalian toxicity (including metabolism), and use and disposition patterns.

EPA believes that this category is not supported for any of the proposed category endpoints, and should be reconsidered because of significant differences in the chemical structures, in the metabolism of at least some of the members, and a lack of evidence of similar mammalian toxicities. For example, piperidine, unlike all the other substances, is not a heterocyclic aromatic compound and is not expected to metabolize in the same manner as pyridine and the other compounds (the submitter notes that the metabolites of piperidine are different from those of pyridine). In addition, the pyridinium salt has a cationic charge and a molecular weight more than double that of pyridine; therefore, it is not expected to behave similarly to pyridine. Also, the structural differences between the nitriles and the alkylpyridines are sufficient to render dubious any toxicity read-across between these structure types.

Considering these dissimilarities, EPA suggests that the substances could be grouped as follows:

1) Piperidine
2) Alkylpyridines: pyridine; 2-picoline (2-methylpyridine); 3-picoline (3-methylpyridine); 4-picoline (4-methylpyridine); pyridine, alkyl derivatives
3) Pyridinium chlorides: 1-phenylmethyl pyridinium chlorides, Et Me derivatives
4) Pyridinenitriles: nicotinonitrile, picolinonitrile

EPA reserves judgement on whether the existing and proposed testing for four members of the alkylpyridines group (pyridine and the three picoline isomers) can be used to read across to pyridine, alkyl derivatives pending additional information on the composition and purity of the alkyl derivatives mixture.

Read-across of endpoint data among chemicals is possible for some endpoints within the two mulltichemical groups but not between any of the groups.

For biodegradation no read-across is possible even within groups because the structural variations in these chemicals may affect the biodegradation potential and rate.

While the submitter did not provide any category justification for ecological toxicity, the observed differences in structure are also likely to lead to differences in ecological toxicity.

Test Plan

Physicochemical Properties (melting point, boiling point, vapor pressure, partition coefficient and water solubility)

For piperidine, pyridine, 4-picoline, and 2-picoline, the data provided by the submitter for these endpoints are adequate for the purposes of the HPV Challenge Program. For 3-picoline, the data provided by the submitter for melting point, boiling point, partition coefficient, and water solubility are adequate for the purposes of the HPV Challenge Program. For nicotinonitrile and picolinonitrile, the data provided by the submitter for melting point, boiling point, and partition coefficient are adequate for the purposes of the HPV Challenge Program.

EPA agrees with the submitter's plan to provide melting point, boiling point, vapor pressure, partition coefficient and water solubility data for pyridine, alkyl derivatives and for pyridinium chlorides.

Vapor Pressure. The estimated vapor pressure data for 3-picoline, nicotinonitrile and picolinonitrile provided by the submitter are not adequate for the purposes of the HPV Challenge Program. Vapor pressures are expected to exceed 10^{-5} Pa for these chemicals, and need to be measured by the submitter following OECD Guideline 104.

Water Solubility. The submitter did not provide water solubility data for nicotinonitrile and picolinonitrile. The submitter indicates only that these two chemicals are "soluble" in water, which is not acceptable for the purposes of the HPV Challenge Program. Furthermore, the HPV Challenge Program requires that water solubilities expected to be greater than 1 µg/L be measured according to OECD Guideline 105. The submitter needs to provide measured water solubility data for these two chemicals following OECD Guideline 105. EPA found a water solubility value of 1.35×10^5 mg/L at 20°C for nicotinonitrile in the 4th Edition of the Kirk-Othmer Encyclopedia of Chemical Technology, 1996 (as cited in HSDB, 2001). EPA recommends that the submitter add this value to its robust summary.

Environmental Fate (photodegradation, stability in water, biodegradation, fugacity)

For piperidine, pyridine, 4-picoline, 3-picoline, 2-picoline, nicotinonitrile, and picolinonitrile, the data provided by the submitter for photodegradation and fugacity are adequate for the purposes of the HPV Challenge Program.

Photodegradation. The submitter did not provide photodegradation data for pyridine, alkyl derivatives, and pyridinium chlorides. As indicated in the Category Justification Section, EPA does not agree with the submitter that these two chemicals can be members of a single category. Therefore, the submitter needs to provide photodegradation data (measured or estimated) for these two chemicals. If the submitter plans to use estimated values for this endpoint, EPA suggests that the submitter enter SMILES structures in EPIWIN that would best represent these two chemicals.

Stability in Water. While EPA agrees that the submitted chemicals are stable in water under environmental conditions and do not hydrolyze, the submitter needs to address this issue in the robust summaries as well as the test plan.

Biodegradation. The biodegradation data provided for picolinonitrile and nicotinonitrile are not adequate for the purposes of the HPV Challenge Program. Literature values were used to demonstrate that both nicotinonitrile and picolinonitrile would undergo moderate rates of biodegradation, but these reports used non-standard methods. The submitter needs to provide measured ready biodegradation data for these two chemicals following OECD Guideline 301.

The estimated biodegradation data provided by the submitter for piperidine are not adequate for the purposes of the HPV Challenge Program. The submitter needs to provide measured ready biodegradation data for this chemical following OECD Guideline 301. EPA located results of a Japanese

MITI test for piperidine (Sasaki, 1978) and recommends that the submitter include this information in its robust summary.

The submitter did not provide biodegradation data for pyridine, alkyl derivatives, or for pyridinium chlorides. The sponsor suggests that the biodegradation endpoint for these substances could be met by reading across from existing or proposed test data. EPA disagrees because the structures of all the sponsored substances differ enough that the read-across approach is not warranted. The submitter needs to provide measured ready biodegradation data for these two chemicals following OECD Guideline 301.

Fugacity. The submitter did not provide fugacity data for pyridine, alkyl derivatives, and pyridinium chlorides. As previously indicated, EPA does not agree that a read-across approach is supportable for the sponsored chemicals. The submitter needs to provide fugacity data for these two chemicals. EPA suggests that the submitter enter SMILES structures in EPIWIN that would best represent these two chemicals in order to develop the fugacity estimates.

Health Effects (acute toxicity, repeated-dose toxicity, genetic toxicity, and reproductive/developmental toxicity)

Adequate data exist for the following chemicals and endpoints:

1) *Piperidine:* Adequate data are available for acute toxicity.
2) *Alkylpyridines:* For pyridine, adequate data are available for acute toxicity, repeated-dose toxicity, and genetic toxicity (gene mutation and chromosomal aberrations). For 4-picoline, adequate data are available for acute toxicity and gene mutations. For 3-picoline, adequate data are available for repeated-dose and gene mutations. For 2-picoline, adequate data were provided for acute toxicity, repeated-dose toxicity, and gene mutations.
3) *Pyridinenitriles:* For nicotinonitrile, adequate data exist for acute, repeated-dose, and genetic toxicity. For picolinonitrile, adequate data exist for acute toxicity.

EPA reserves judgement on the gene mutations data for piperidine pending information on whether positive controls were used in the mammalian cell forward mutation assay and/or an explanation as to why the bacterial reverse mutation assay conducted by Florin et al. (1980) used a dose that is 20 times lower than the highest dose recommended in OECD TG 471. All other gene mutation studies for piperidine used non-standard bacterial strains.

EPA reserves judgement on the adequacy of the acute toxicity data for pyridine, alkyl derivatives, and for pyridinium chlorides, pending additional information on the identity and purity of the chemicals used and how the tested substances are relevant to the sponsored chemicals. If these data are judged to be inadequate, additional testing is needed for these chemicals.

The submitter proposes that tests according to OECD TG 421 be conducted on pyridine and on nicotinonitrile. For pyridine, EPA recommends testing in mice rather than rats because of their greater sensitivity to pyridine. Because some data are available for nicotinonitrile and because acute toxicity testing indicates greater toxicity of picolinonitrile compared with nicotinonitrile, EPA recommends that the submitter conduct a repeated-dose/developmental/reproductive toxicity screening test on picolinonitrile instead of nicotinonitrile.

To address other deficiencies, the submitter needs to conduct a repeated-dose/developmental/ reproductive toxicity screening study (OECD TG 422) and testing on chromosomal aberrations for piperidine. In addition, EPA recommends that OECD TG 422 and genetic toxicity testing (both gene mutations and chromosomal aberrations) be conducted on pyridinium chlorides.

EPA believes that a read-across approach can be applied from the existing, proposed, and EPA-recommended testing in the alkyl pyridine group to the data gaps within this group, pending information on the composition of pyridine, alkyl derivatives. Existing data on nicotinonitrile and the EPA-recommended study on picolinonitrile can be used to read across between these two chemicals.

Acute Toxicity. The acute toxicity data have several deficiencies, including limited method details, no information on purity of the chemicals, limited number of animals used, or use of a single sex. However, in most cases, multiple studies are available for a single chemical, or a read-across approach can be used from other chemicals in the same EPA-defined chemical group.

The relevance of the alkylpyridine derivatives oral toxicity study is not clear because the chemical tested for this endpoint was a mixture of pyridine, HCl, methanol, surfactant, and water. Similarly, for pyridinium chlorides, a mixture pyridinium chlorides, surfactant, isopropanol, water, and thiourea was used. No percentage composition data were provided in either case.

Repeated Dose Toxicity. For piperidine, the 5-month inhalation repeated-dose toxicity study is limited because only two dose levels were used. Even though effects were observed, a complete dose-response cannot be determined using only two dose levels. For this reason and the limited assessment of reproductive parameters, EPA recommends conducting a repeated-dose/developmental/reproductive toxicity screening study (OECD TG 422) for this chemical.

Reproductive Toxicity. For piperidine, the reproductive toxicity evaluation is limited to male sex organs and functions from the repeated-dose toxicity study, which is also limited in the number of dose levels used. Therefore, EPA recommends conducting a test according to OECD TG 422.

Ecological Effects (fish, invertebrates, and algae)

To adequately address data needs, the submitter needs to provide data on the following chemicals: 1) piperidine; 2) pyridine, alkyl derivatives; 3) pyridinium chlorides; and 4) picolinonitrile for all ecological endpoints.

The submitter provided adequate data for acute fish toxicity for picolinonitrile in the current submission; however, daphnia and algae tests need to be conducted. For piperidine and pyridinium chlorides, all endpoints need to be tested. For pyridine, alkyl derivatives, an algal test needs to be conducted. Also for this substance, detailed information on the composition and purity of substance tested on the fish and daphnia studies is essential for determining data adequacy. Otherwise, tests on these endpoints need to be conducted for this chemical.

Specific Comments on the Robust Summaries

Health Effects

Genetic Toxicity (Gene Mutations). The robust summaries for *in vitro* gene mutation studies for piperidine, 4-picoline, 3-picoline, 2-picoline, and pyridine were often missing statistical methods, the number of replicates, culture conditions, occurrence of cytotoxicity, and whether a particular OECD guideline was followed.

Robust summaries were submitted for two sex-linked recessive lethal mutation studies for pyridine. One or both studies lacked the purity of the test substance and the method followed.

Genetic Toxicity (Chromosomal Aberrations). Some of the summaries for pyridine were missing the statistical methods used, the test substance purity, the number of replicates used, culture conditions, occurrence of cytotoxicity, and whether a particular OECD guideline was followed.

Ecological Effects

For 4-picoline, 3-picoline, and pyridine, the purity of the test substance was cited as “Not stated” in some of the robust summaries. Also, for pyridine, alkyl derivatives, the purity and chemical identity of the test substance were not addressed.

The limited study data did not allow a determination of the adequacy of these studies. The submitter

needs to provide the missing study details.

Followup Activity

EPA requests that the submitter advise the Agency within 90 days of any modifications to its submission.

References

Florin, I., L. Rutberg, M. Curvall, and C.R. Enzell. 1980. Screening of Tobacco Smoke Constituents for Mutagenicity Using the Ames Test. Toxicology. 18: 219-232.

HSDB (Hazardous Substances Databank). 2001. 3-Pyridinecarbonitrile; CAS 100-54-9. Available at: http://toxnet.nlm.nih.gov.

Sasaki, S. in: Aquatic Pollutants: Transformation and Biological Effects. Hutzinger, O.; Von Letyoeld, L.H.; Zoeteman, B.C.J. (Eds), Oxford: Perfamon Press, pp 283-89.